A SUDANESE MADUROMYCOSIS

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INTRODUCTION

In 1842, Gill, of Madura, in his Dispensary Report, drew attention to a disease of the foot which produced marked deformity and fungoid excrescences discharging an offensive ichorous fluid, while internally it caused destruction of the joints, cartilages and ligaments, the diseased tissue resembling fibro-cartilage.

In 1846, Colebrook, also of Madura, again reported upon this disease, which he said was commonly known in some parts of Southern India as '*Madura Foot*.'

In 1860, Vandyke Carter applied the term 'Mycetoma' or fungus tumour (*μύκης* a fungus and *ὀίδημα* a tumour) to that variety of Madura foot which contained black granules, and one year later included under this name the white or yellow variety of the same complaint with which he had become acquainted.

Thus almost from the commencement of scientific enquiry into the disease, the black and white varieties were grouped together under the same term, and it appears advisable to continue to do this at the present time, although the name now embraces a large and varied series of different pathological conditions.

With this proviso Mycetoma may be defined as:—

> 'All growths and granulations producing enlargement, deformity and destruction in any part of the body of man, brought about by the invasion of the affected area by certain species of fungi belonging to different genera which give rise to variously coloured and shaped bodies called "grains," which are formed of hyphae with or without chlamydospores, and are found either embedded in the pathological tissue forming the growths and granulations or escaping freely in the discharge from the diseased area.'

The presence of definite grains separates the Mycetomas from the pseudomycetomatous conditions caused by Sporotrichosis, Framboesia Tropica and Angiokeratoma.

Such a definition, however, covers very wide pathological and aetiological fields, but thanks to the labours of Pinoy, the diseases classified together under the general term 'Mycetoma' may be divided into two very distinct groups, viz.: *The True Mycetomas* and *The Actinomycoses*.

But confusion may arise between the terms '*Mycetoma*' and '*True Mycetoma*,' and, therefore, to prevent this, we suggest the word '*Maduromycosis*' instead of True Mycetoma. The word 'Maduromycosis' appears to us to be suitable because the disease was known as '*Madura Foot*' long before Carter introduced the term Mycetoma.

The Mycetomas are therefore divided into:—

A. *The Maduromycoses* are those forms of Mycetoma with grains composed of large segmented mycelial filaments possessing well defined walls, and usually chlamydospores.

B. *The Actinomycoses* are those forms of Mycetoma with grains composed of very fine non-segmented mycelial filaments, in which usually the walls are not clearly defined from the contents, and in which chlamydospores are absent.

The Maduromycoses may be classified according to the colour of their granules into:—

I. The Black Maduromycoses.
II. The White Maduromycoses.

Judging by Balfour's description of a Mycetoma which contained very hard spherical grains of a brick-red colour, in which Archibald found appearances suggestive of an aspergillar infection, there must be a third class, viz.: *The Red Maduromycoses*, but, if so, this division still requires detailed investigation.

We propose in the present paper to restrict our remarks to the Black Maduromycoses of which we have met with examples in the Anglo-Egyptian Sudan, but before so doing we desire to review the work which has already been performed with regard to this subdivision of the Mycetomas.

HISTORICAL

In order to make clear the points which we desire to raise later on, it is convenient to subdivide the history of the Black Maduromycoses according to the continents in which they have been found, i.e., into:—

(*a*) The Asian Black Maduromycoses.
(*b*) The European Black Maduromycoses.
(*c*) The American Black Maduromycoses.
(*d*) The African Black Maduromycoses.

(*a*) *Asian.* Excluding some ancient references discovered by Collas, to which Corre has drawn attention and which will be considered when we discuss that author's writings, the history of the Black Maduromycoses commences in 1845 in India, where Garrison-Surgeon Godfrey, in his Departmental Report of the Public Dispensary at Bellary, described the occurrence of a considerable black deposit, much resembling fragments of coal, in a foot which had been amputated because it was affected by a disease which was commonly known as '*ulcus grave*,' because the ulcers and sinuses produced such a serious condition that amputation became necessary. This disease he had described in the same report for the preceding year, designating it '*morbus tuberculosis pedis*,' because, though he recognised it to be dissimilar from other recorded

diseases, he looked upon it as a local tubercular affection, and, influenced by this view, he considered the black particles mentioned above to be accidental, and not essential parts of the disease. He also mentions that it was known to the natives as *Ghootloo Mahdee*, from the tubercular irregularities being supposed to resemble eggs.

This first case of Black Maduromycosis occurred in a native aged about 30 years, and had existed four to five years before amputation was performed. The morbid appearances are described as being similar to those fully set forth in his 1844 report, with the addition of there being in this instance one cyst (or excavated tubercle) containing melanotic matter about the size of a small walnut and extending from the plantar to the dorsal aspect of the foot between the metatarsal bones of the great and second toes, which were in part absorbed. The integuments were not involved in this mass, which when recent had an angular and brilliant black appearance much resembling a fragment of coal, and was considered to be an accidental product in this peculiar case.

Carter says that the second volume of the *Indian Annals* (probably dated about 1849) on page 706 contains an account of dark granular or black gritty particles being found among the bones and in the sinuses of a diseased foot, but we have been unable to refer to this work, and are ignorant of the name of the discoverer and of the date and place in which this observation was made. The particles in question were examined microscopically, and were believed to consist entirely of dried blood, a belief which lasted for many years.

It may perhaps be advisable at this point to draw attention to the fact that Ballingall's celebrated observations do not refer to the black but to the yellow variety of Mycetoma, and hence do not enter into this history.

Sub-Assistant Surgeon Bazonji Rustonji (1858), of the Bhoo's Dispensary, in the Province of Kutch, drew attention to the fact that there were two forms of the disease, viz.: one in which there was no granular deposit, but only a substance dark in colour and soft and thick in consistence; while the other showed small, soft, yellowish granules. This is the first occasion, as far as we know, when a differentiation was made between the Melanoid and the Ochroid varieties of the disease, but Rustonji did not recognise the fungal nature of the bodies in question.

Eyre (1860) states that in every foot examined by him there were numerous minute tubercles resembling fish roe, which were found lying beneath the muscles and extending from the bones to beneath the skin, with nodules of the same appearance and often black in colour. This paper deals with the external characters of the disease, its previous history, natural course, morbid anatomy, aetiology (which was doubtfully thought to be somewhat tubercular) and treatment.

In 1860, Vandyke Carter began that series of classical observations upon the Black and Yellow forms of Madura Foot, which he continued until 1874, and during which he firmly established the fungal nature of the disease.

His first paper (1860) was entitled '*On a new and striking form of fungus disease affecting the foot and prevailing endemically in many parts of India.*' In his second publication (1860) he clearly differentiated between the white or ochroid division of the Mycetomas which to-day we call '*Actinomycosis*,' and the black or melanoid variety which we now name *Black Maduromycosis*. He demonstrated that the black grains were of true vegetal nature, with a black friable mind composed of clear orange tinted, ovoid or angular cells and beaded fibres closely arranged so as to form a compact structure, and in addition larger vesicular bodies (seemingly comparable to gemmules or sporangia), which he thinks may arise at the extremities of the compressed beaded fibres by gemmation and expansion. The pale reddish-brown central part of the larger sclerotes was composed of slender, pale, flattened and branching fibres arranged in bundles and intermixed with numerous granules and a few large beaded fibres, the septa of which were sometimes absent.

He placed some black particles, taken from a foot, on cotton soil moistened with animal juices and enclosed in a stoppered bottle, which he left unopened for $2\frac{3}{4}$ years, when he found a thin reddish film had appeared. Other black particles sown on rice paste for the same length of time remained unchanged, but on opening the bottle a red mould speedily made its appearance.

With reference to this mould, he says: 'It had not, however, a clear connection with the fungus particles, but seemed to spring up independently of them upon the rice whenever this was exposed to the air.'

This statement is of importance, as he grew a fungus from the white variety which was pink in colour, and produced sporangia resembling those of a species of the genus *Mucor* Micheli 1729, but differing therefrom in the absence of a columella which should have brought it under the genus *Mortierella* Coemans 1863, but Berkeley, who examined the growths from a botanical point of view, classified it under the genus *Chionyphe* Thienmann 1839, calling it *Chionyphe carteri* Berkeley 1862, and defining it as :—'Hyphasmate ex albo flavorubroque, sporangiis demum coccineis, sporis breviter fusiformibus.'

The genus *Chionyphe*, however, was never recognised by mycologists generally, as its species came under the genera *Mortierella* or *Mucor*, while *Chionyphe carteri* was most undoubtedly a contamination as its connection with the black or white grains was never proved, as we have noted above with regard to the former.

Thus we may conclude that although Carter gave the first proof of the parasitic nature of the grains, he was unable to produce growths by cultivation from either the black or the white varieties.

In 1860, Minas wrote upon *Keerccnagoah* of the Foot, as seen in the Punjaub. The term used is a vernacular word signifying worm disease. He states that the characteristic symptom of the complaint is gradual enlargement of the foot, usually starting with a swelling in the sole associated with the presence and constant discharge of small particles, either soft or black and hard, from fistulous openings.

Collas (1861) described Black Maduromycosis as seen in Pondichèry. He recognised the little bodies of blackish or reddish brown colour which in their clearer parts seemed to be formed of small transparent cells, which he could not sufficiently study. He called the disease '*Dégéneration endémique des os du pied.*'

H. J. Carter (1862) came to the conclusion that the fungus of Black Maduromycosis was nearly allied to *Mucor stolonifer* Ehrenberg, 1818, the spores of which in an amoeboid state he considered entered the body through the sudorific ducts. Berkeley (1862) mentioned the fungus in question; he gave it the name *Chionyphe carteri*, a nomenclature which he subsequently repeated (1865).

In 1867, Moore reported an important early case in which he effected a cure by cutting and scraping away all the diseased tissues, and he agumented this in 1873 by recording two more cases of a similar nature, treated in the same way with a like result.

In 1870, Holmsted, of Hydrabad, Sind, found a thorn of irregular shape and half an inch long in a case of Black Mycetoma, in which it had been embedded for two years. In the same year, Bristowe described and figured the fungus seen in the black particles of a foot from a case of Black Maduromycosis amputated in Cantoor, and demonstrated to the Pathological Society of London by Tilbury Fox. Bristowe's descriptions and figures are excellent, and amply confirm Vandyke Carter's work. Thudichum chemically examined the black pigment of this case, and showed that it was not derived from blood.

Hogg (1872) described a Black Maduromycosis from India, in which he was able to observe the fungal threads and to resolve them into jointed dissepimented cells, some branching out and attaining a considerable length, while others terminated in an enlarged ovoid head. He, however, believed that the fungus was a secondary product, which might greatly aggravate but did not originate the disease, and suggested that it might be introduced at the time of the first accident when the foot was struck against a stone, or by the poultices used as treatment in a later stage.

Vandyke Carter (1874) published his monumental and classical work '*On Mycetoma or the Fungus Disease of India*,' which concluded his long continued labours at this complaint.

Lewis and Cunningham (1875) admitted the fungal nature of the black particles, but not of the yellow granules. They showed that *Chionyphe carteri* had nothing to do with black or yellow grains.

In 1876, Berkeley came to the conclusion that *Chionyphe carteri* had nothing to do with Mycetoma, a point which can be easily judged from the passages quoted above.

Notwithstanding all these researches, a great deal of confusion still existed with regard to the disease, which can be judged by a study of Fox and Farquhar's (1876) report. It was admitted that the black granules were fungal in nature, but it was contended that they were not causal in effect, because all the essential features of Mycetoma were found to be present without any black fungal particles, and because there was not sufficient evidence forthcoming at the time in proof of the vegetal character of the yellow grains, which were believed to be essentially fatty in nature. It was,

however, admitted that Moore's observation showing that the black variety could be cured by excision of all the particles at an early stage of the disease was a strong argument in favour of the parasitic nature of Mycetoma.

Though Carter had found black, yellow or white, and red grains, still the general belief was that these were one and the same process, and, moreover, observers of this period must have seen the Pseudo-Mycetomatous conditions mentioned above, because competent workers appear to have met with cases in which they were unable to find any grains, although the clinical appearances resembled Mycetoma.

Corre (1883) placed in order, completed and revised the notes of researches made by Collas since his publication, already mentioned, in 1861. In these notes, which were published after his death, Collas desired his previous name for the disorder to be altered to '*La Maladie de Ballingall*,' and states that the earliest references to the disease with which he is acquainted can be found in Waring's paper, and in one of the sacred books of the East which he calls '*Vaweda*' (Ushta wunga hrethayum), which appears to us to be the '*Atharvavéda*.' In this latter work, '*Slipatham*,' or elephant foot, is distinguished from '*Padavalmicum*,' which refers to an incurable malady of the foot associated with swelling and the formation of fleshy tumours, from which, about a year after the appearance of the first symptoms, there exudes a peculiar fluid. He also points out that the words *Perikal*, *Anaikal* (Tamil)—this means Cochin leg—*Slipada* (Bengalese), *Hatty-ka-poung* (Dekkan), are applicable to Elephantiasis as well as to Madura Foot, and, therefore, should not be specially applied to the latter, as they really mean the 'leg of an elephant.' In Ballary, he says, the disease was called '*Gootloo Mahdee*,' because the swellings on the foot were thought to be like eggs; while in Rajputana it was called '*Kirinagras*,' or the dwelling house of worms, because the sinuses were considered to be like the cavities often occupied by the larvae of flies. He also says that in 1714 a missionary described under the name of *Fourmilière des vers* a disease of Pondichèry which was incurable, and in which numerous ulcers intercommunicated by means of small canals full of worms, which were peculiar in that if one closed

another opened. This information Collas obtained from Volume II, page 167, of a book published in Paris in 1812, and entitled *Mémoires sur les moeurs et coutumes de l'Inde par un missionaire*. Collas also points out that in 1806 Heyne saw the brother of a Rajah at Cuddapah in Hyderabad with a foot in a leprosy state, but which was considered to be distinct from leprosy, although it was not known what the nature of the disease might be. Collas thinks that this must have been Mycetoma, and draws attention to Brett's *Sarcomes adipeux*, in which he says it is difficult not to recognise Ballingall's disease.

With reference to the above names, it will be noted that they apply to any form of Mycetoma, and not especially to Black Mycetoma. The name *Ballingall's Disease*, in our opinion, is not applicable to the Black Mycetomas, because, as already indicated, he was not acquainted with the disease.

In 1886, Carter gave up his pink mould, and drew attention to the similarity between the fungus of Actinomycosis and that of Mycetoma.

Kanthack (1893) studied both the Yellow and Black Mycetomas, and came to the conclusion that the former agreed morphologically and structurally with Actinomycosis, but with regard to the black grains his position was curious, for although he found them to consist of an olive-brown, glassy or finely granular material, in which hollow filaments, radially arranged, were embedded, still he regarded these as degeneration changes, and sought to prove that the granules were an organism allied to the Actinomycosis fungus which he had found in the yellow variety. Thus, like Vandyke Carter, he believed both varieties to be fungal in nature and to be caused by the same fungus, but he attempted to show that the fungus of the yellow variety existed in the black, while the former observer believed the reverse to be true. He named the fungus *Oospora indica* Kanthack 1893, and distinguished the two varieties as *O. indica var. flava* and *O. indica var. nigra*. Unna, however, to whom he sent specimens, did not make this error, but says: 'A whole series of important distinctions separate the two fungi, and there is no question of their identity.'

Boyce and Surveyor (1894), in a most important paper, first definitely proved that the fungi existing in the black

and yellow varieties were quite different, and thus definitely established the two main divisions of Mycetoma, which to-day we call Madurom ycosis and Actinomycosis. They showed that the black grains were composed of a large, septate, branching fungus embedded in a brown pigmented ground substance, which was readily bleached by Eau de Javelle. They did not observe spore formation, nor was cultivation attempted.

In the same year, Boccaro also differentiated between the white and the black varieties of the disease.

Chatterjee (1911) observed that grains placed in agar and glucose agar tubes increased in size some seven to eight times in four days, and were surrounded by fine hair-like structures which were composed of delicate branching mycelial threads, which were seen to come from the thick black threads. On potato, the growth was dry and black. In broth, small white colonies composed of radiating threads were found sticking to the walls of the tube. No diffuse growth was seen, nor did any scum form on the surface. Animal experiments were negative.

Mackenzie, in the same year, appeared to obtain similar cultures on agar; at first the growth was white and translucent, with radiations from the centre, later it became greyish yellow, there being a central granule surrounded by a clear zone and an indented margin. After a week the colony became a deep mahogany, and under the microscope exhibited mycelial structures.

Semon (1915) reported a case of Black Maduromycosis which occurred in a native Indian soldier serving in France. He left India about October, 1914, and in January, 1915, he injured one of his feet by the fall of an ammunition box. The patient attributed the disease to this cause, but Semon considers, probably correctly, that he must have been infected before leaving India. A typical Mycetoma developed in about six months, and the pus contained black particles in which a central mass of mycelium obscured entirely by black pigment could be made out, but no proper demonstration of the fungus *in situ* could be made. The foot could not be amputated, but sections were made of some of the tissue which showed marked vascular hypertrophy, polymorphonuclear, plasma and connective tissue cells, but no endo- or periarteritis and no giant cells.

Excellent growths were obtained at 35° C. on agar agar, maltose agar and Raulin's fluid. With great kindness, Semon sent us one of these growths, which is depicted in fig. 33.

The fungus first formed a central black portion with a peripheral zone of white or grey, which in the course of ten days, or less, became black. The cultures soon became pleomorphic, and lost their definite characters. They showed no chlamydospores when examined microscopically. We have been able to grow this fungus, and can confirm Semon's statements.

Treatment was attempted by giving injections of boiled and unboiled cultures in Raulin's medium, but without effect. Remittent fever, with pain and inflammation of the foot followed treatment by iodide of potassium in 20 grain doses three times a day, while X-rays were useless for diagnostic or therapeutic purposes.

At the present time it is customary to consider that there is only one organism causing this Asian variety of Maduromycosis and that this is the same as *Madurella mycetomi* Laveran 1902, the origin of which we propose to discuss below.

(*b*) *European.* For a long time Maduromycosis was considered to be essentially a tropical disease, but though this in part holds good, still there are an increasing number of cases being reported in Europe.

The first Italian Black Maduromycosis was discovered in 1886 by Bassini in Padua, in a man who had never left Italy, and who had pricked his foot with an iron pitchfork while working in a cattle stall in 1885. The wound healed, but little by little pain and swelling developed in the region of the healed wound, and these symptoms spread so rapidly that in about seven to eight months the patient was unable to walk, and finally consented to amputation in November, 1886, after which a cure was effected. Bassini gave an excellent and well illustrated account of this case in 1888, recognising that the black grains were composed of radially arranged septate hyphae, 4 to 6 microns in diameter, showing irregular swellings and being embedded in a dark brown matrix. He also observed strands of hyphae, not enclosed in grains, also embedded in a brown matrix. Spore formation was not seen, and cultivation was not attempted. Pepere thinks that, without entirely excluding other possibilities, Bassini's parasite may have been an Aspergillus or some closely allied fungus.

Köbner (1891) appears to have reported a case of Black Maduromycosis from Italy, which was thought to be due to a Mucor or an Aspergillus, but we have been unable to refer to this paper, which is mentioned by Kanthack.

In 1906, Paolo Bovo described a Mycetoma of the foot in an old man in Genoa. The disease was characterized by superficial nodules associated with a single ulcer and by invasion of one of the glands in the groin.

The grains, which were black, varied from a millet seed to a pea in size, and were composed of a black feltwork of filaments and spores, both of which were easily observed. Bovo thought that it might be an Aspergillus, but no cultures were made, and very curiously the author insists upon the absence of any inflammatory process and that the single ulcer was due to mechanical attrition.

Brumpt examined Bovo's specimens, and came to the conclusion that it was probably a Madurella, and hence it is known as *Madurella bovoi* Brumpt 1910. Pepere, however, says that there can be no doubt as to the aspergillar character of the granules, and as the genus Madurella has now been more accurately defined by Brault and Pinoy, it is better to leave this fungus unclassified.

In 1906, Busacchi described a Black Maduromycosis having some analogy with that of Bassini, and occurring in a peasant aged 36 at Cremona. He cultivated the parasite, but he has not yet fully reported upon its nature. It grew well on any liquid or solid media, forming a black or yellowish layer. It was a branching fungus, forming chlamydospores, which he does not consider to be an Aspergillus, but thinks that it is a Streptothrix, by which he does not mean that it is an Actinomycosis, while he mentions the fact that he had met with a similar case in 1896.

From Pepere's article we gather that Schmincke, in 1910, observed a case of Black Mycetoma somewhat analogous to Bassini's, but found in Kissingen.

A new phase in the history of the European Black Maduromycosis is opened by Pepere's (1914) exceedingly able work on a form due to a Monosporium.

Prior to this, Tarozzi (1909) had published a paper, in which he described a white Maduromycosis of the foot in a butcher of Ibono, in the Province of Cagliari, in Sardinia. Radaeli in 1911 gave an account of a case of White Maduromycosis

of the foot occurring in a peasant living in Montemurlo, near Florence, in which the causal organism was recognised to belong to Bonorden's genus *Monosporium*, and was eventually called *Monosporium apiospermum* by Saccardo, in 1911. We have been unable to refer to Tarozzi and Radaeli's original papers, but we have read their controversial papers, and there appears to be no doubt that both met with the same parasite causing White Madurомycosis in Italy and Sardinia.

Pepere's Black Madurомycosis occurred in a peasant, aged 33 years, who lived at Domusnovas, in the Province of Cagliari, in Sardinia. This case was most carefully investigated in every way. Cultures were made on various media and photographs taken therefrom, successful inoculations into the anterior chamber of the eye in guinea-pigs were performed, complement fixation was studied, while the mycology of the fungus was most ably investigated.

The causal organism closely resembled *M. apiospermum*, except that it caused a Black and not a White Madurомycosis, and Pepere, correctly in our opinion, considered that this parasite must be different from *M. apiospermum*, and therefore named it *M. sclerotiale* Pepere 1914. He also used the terms *Monosporium sclerotiale* (*seu nigricans*).

When we compared Pepere's photograph of the fungus with Bonorden's drawings of various species of Monosporium we were much impressed with the differences, and we were therefore by no means surprised to read in Pepere's paper that Saccardo proposed to separate *Monosporium apiospermum* from Bonorden's genus and to make a new genus *Scedosporium* for it. This new genus would, of course, contain Pepere's species also, but, unfortunately, we are unable to give a definition of this new genus, as we have been unable to obtain Volume XXII of Saccardo's Sylloge Fungorum.

(*c*) *American.* The next type of Madurомycosis comes from America, where Wright (1898) described a case of black Mycetoma which occurred in an Italian woman, aged 26 years, who had resided in Massachusetts for several years. The disease was first noticed some six months before she applied for treatment, and was confined to the base of the second and third toes on the plantar aspect, which area was swollen and contained a small sinus from

which exuded a dirty greyish fluid containing black, hard, irregular granules, like grains of gunpowder, which were composed of ovoid or rounded translucent bodies of varying sizes, closely packed together, and having a homogeneous interior or containing a few refractile granules. Septate branching hyphae sometimes showing dilatations were also seen, while the periphery of the grain was made up of closely set radiating hyphae, showing more or less marked swellings. All these structures were embedded in a brown refringent substance.

On cultivation in broth, puff-balls were produced which eventually filled the fluid. This finally acquired a deep coffee brown colour, while a film was formed on the surface.

In potato infusion it grew as in broth, but formed no surface growth, and in old cultures gave rise to black sclerotia, 1 mm. in diameter, composed of short thick segmented hyphae and spherical or polyhedral cells with black walls.

On potato it formed a dense, spreading velvety pale brown layer with a white periphery, on which small globules of dark coffee coloured fluid were seen, while the medium became dark brown and very moist.

On agar-agar and glucose agar slants it formed a meshwork of greyish filaments, and in old cultures the black sclerotia appeared, while in stabs there were only surface growths. Spore formation does not appear to have been observed in any of the cultures. Some authorities have considered that Wright's organism was a contamination, but a study of his photographs and description has convinced us that this is not so, and we are inclined to believe that he commenced the cultivation of either a Madurella or a fungus like the one we shall describe, which may or may not be different from those obtained in other places. It is curious that the case should have occurred in an Italian woman, but the chance of the infection having been acquired in Italy appears to be remote, as she had lived in America for several years.

It is not definitely stated that she had never visited Italy during that period, but Wright was well acquainted with Bassini's researches, and would probably have drawn attention to the possibility of infection during such a visit if it had been known to have taken place.

We therefore, in the absence of full details, must assume that this Italian woman became infected in the United States and not in Italy, and that the Madurомycosis was American and not European in origin.

de la Hoz (1905) published a thesis on the 'Pathogenic Fungi and Mycoses of the American Continent, but did not increase the number of observations with regard to Madurомycosis.

Seheult (1916) described a case of Black Madurомycosis which occurred in Trinidad, in an East Indian immigrant, who had arrived in the colony in 1899, and whose foot was injured, apparently without causing a wound, by the fall of a cocoa pod in 1908, i.e. nine years after his arrival in the colony.

Three years later, and twelve years after his landing, the foot began to swell and sores appeared upon the instep, and eventually a typical Black Madurомycosis developed, which was said by Balfour to resemble a type found in the Anglo-Egyptian Sudan.

Four years after the commencement of the symptoms, and sixteen years after his landing, the foot was amputated.

This is the first case of Black Madurомycosis to be recorded in the West Indies, and is the second, as far as we know, to be published with regard to the American Continent, though it is possible that we have missed records owing to the limited literature in Khartoum.

It appears to us to be of peculiar interest, because with Semon's and Wright's cases it forms a series of greater and greater possible latency.

I. *Semon's Case* occurred in France, in an Indian soldier who had left India about three months prior to the accident and nine months before the typical Mycetoma developed.

II. *Wright's Case* occurred in the United States, in an Italian woman who had left Italy an indefinite number of years previous to the onset of the disease, which was treated in the same year in which it was first noticed, and after only six months' growth.

III. *Seheult's Case* occurred in the West Indies, in an Indian who had left his native country twelve years before the onset of the disease.

These cases have compelled us to seriously consider the question of latency, and we find ourselves unable to say how long this can

last, and therefore it is possible that Cases I and III were originally infected in India, while Case II may have acquired the infection in Italy.

(*d*) *Africa.* In 1901, Brumpt, Bouffard and Chabaneix wrote an account of a case of Black Mycetoma which they observed at Djibouti. In the following year, the organism found in this case was studied by Laveran, who gave it the name *Streptothrix mycetomi* Laveran 1902. Brumpt also found the same organism in a Maduromycosis in the centre of Somaliland, and also in an amputated foot sent from Madagascar.

Bouffard, in 1905, reported the presence of the same disease in Senegal and in the French Sudan.

In this variety the grains are black or deep brownish red, and always hard, and generally small, from 1 to 2 millimetres in diameter, when single, and not in accumulated masses. The surface is irregular, with projecting points. On clearing with Eau de Javelle the fungal elements can be clearly seen.

Brumpt (1905) formed a new genus, '*Madurella*,' for this fungus, defining it as follows:—

> 'Mucedine with white thallus, living parasitically in various animal tissues (bone, muscle, connective tissue), possessing during its vegetative life, filaments with a diameter greater than one micron, and even reaching to 8-10 microns. These filaments are septate and branch from time to time, they secrete a brown substance. When old, these filaments form a sclerote, and their walls sometimes become impregnated with a brown pigment. In this sclerote, there are a number of rounded corpuscles from 8-30 microns in diameter (chlamydospores).'

The type species is the organism called *Streptothrix mycetomi* by Laveran in 1902, which therefore becomes *Madurella mycetomi* (Laveran 1902), and which was first cultivated by Brault (1911) in material from Algerian cases.

This form of Mycetoma was reported by Balfour (1911) to be present in the Anglo-Egyptian Sudan.

It is generally assumed that this and the Asian, together with the American type, are one and the same disease, but this still requires proof.

In 1908, Nicolle and Pinoy described a Maduromycosis which they found in Southern Tunisia, near the Oasis of Tozeur, with hard dark brown grains about the size of a pin's head; in which segmented and ramified hyphae about 1 to 4 microns in diameter were seen,

as were rounded bodies arranged in chains and resembling the mycelial spores of a Trichophyton, the whole being embedded in a brownish cement substance. Cultures were obtained at 35° C., and the growths were identical on maltose agar, glycerine agar, potato and carrot, and all the media became pigmented black, due to a tyrosinase produced by the fungus, while the colonies which developed in twenty-four hours at 37° C. were white. Microscopically, the growths showed the 'favic nails' so commonly met with in cultures of *A. schoenleini*. The authors looked upon the organism as belonging to the genus *Oospora* Wallroth 1833, with which Vuillemin considers *Achorion schoenleini* Lebert 1845 should be classified. Its name, therefore, became *Oospora tozeuri* (Nicolle and Pinoy 1908).

Inoculation experiments were unsuccessful in the rabbit, the guinea-pig, and the monkey, but two successful infections were obtained in pigeons.

Brumpt, however, considers the fungus to be a *Madurella*, and therefore its name becomes *Madurella tozeuri* (Nicolle and Pinoy 1908).

Brault (1911 and 1912) cultivated the fungi *Madurella mycetomi* and *M. tozeuri*.

The former grew at 20° C. and 37° C. on broth, various agars, potato, carrot and some vegetal liquid media.

In the liquid media the growth appeared as a whitish grey puff-ball, which later became yellowish or brownish, while the medium remained clear and the growth fell to the bottom of the tube.

On solid media it formed a greyish white, duvet-covered* growth, which possessed a central button surrounded by a radiation, and later, when the culture was drier, the medium became coloured.

Glycerine agar was best, as the growth thereon was luxurious, and when old became yellowish in colour while the medium showed a caramel tinge in its entirety.

Glucose glycerine agar produced a growth of the colour of touch-wood. This culture is thrown into black wrinkles, producing an appearance seen on some sea shores.

When the growths of *M. tozeuri* were compared with those of *M. mycetomi* a number of differences were observed.

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* Down-covered.—Eds.

The cultures of *M. tozeuri* grew more quickly, were more luxuriant, and were white, resembling powdered flour. Those of *M. mycetomi* were more discrete grey, 'duveteuse,' radiated, and sometimes showed concentric circles and disassociated more easily than the preceding.

Old cultures on glucose agar or on glycerinated glucose agar were quite different in the two species.

On carrot, *M. tozeuri* attained a deeper brownish yellow colour, while in old cultures on this medium it produces spores in a manner resembling an Oospora.

Pinoy, in his remarks upon the mycology of these two species, says that Brault's *M. mycetomi* very closely resembles that isolated by Nicolle under the name *Oospora tozeuri*. Its filaments are 2 to 8 microns in diameter and do not possess apparatus for fructification, reproducing by a breaking up of the hyphae of the thallus into articles 5 to 10 microns in length, which divide into two spores. These spores are of the same diameter as the hyphae from which they arise, varying from 2 to 5 microns, while the membrane becomes yellowish with age. In addition, chlamydospores can be observed forming at the end of the filaments more or less like favic nails. The spores of *M. tozeuri* are smaller, but are formed in the same manner.

On Sabouraud's gelatine, *M. mycetomi* gives rise to black sclerotes in the depth of the medium. These are very numerous, measure half to one millimetre in diameter, and are composed of hyphal segments more or less cylindrical. Sometimes the sphere attains a diameter of 10 microns and usually contains only one nucleus, but, though studied for a long time, these sclerotes were never observed to have any higher form of fructification. In *M. tozeuri* it is very rare to see the formation of sclerotes, which takes place on the surface of the medium.

On the bases of the researches on *M. mycetomi* and *M. tozeuri*, Pinoy classifies the genus Madurella as follows:—

Genus *Madurella* Brumpt 1905

Fungi: sterile with septate filaments, reproducing by fragmentation of the thallus. The spores are produced secondarily by binary division of the articles formed. They produce Black Mycetomas in man. They grow well at 37° C.

He further differentiates the two species *M. mycetomi* (Laveran 1902) and *M. tozeuri* (Nicolle and Pinoy, 1908) as follows:—

Madurella mycetomi (Laveran 1902)

Mycelium greyish white, when old, yellowish and darkening the media in sugar cultures. Spores varying in dimension from 2 to 5 microns. Sclerotes black and sterile, with a diameter from ·5 to 1 millimetre, formed in the depths of the medium in cultures. Can invade the skin, bone, muscles and connective tissue of man, giving rise to black grains which are small, hard, round and more or less warty, and which morphologically resemble the sclerotes formed in the cultures. Up to the present the inoculation into animals is negative. Very widely spread in Africa. Isolated by Brault from a Mycetoma with black grains in Algeria.

Madurella tozeuri (Nicolle and Pinoy 1908)

Mycelium white, becoming yellowish with age and darkening the medium in sugar cultures. Spores generally small, 2 microns or sometimes even 5 microns in diameter. Sclerotes are only rarely produced, and then they appear on the surface of the medium. Occasionally it gives rise to a Mycetoma in man, in which it forms black amorphous grains which are often made up of mycelial rings enclosing some degenerate cellular elements which are impregnated with the pigment of the fungus, and also of small diffuse masses formed solely by the filaments of the fungus which have a yellow membrane. Inoculation into pigeons positive. Isolated by Nicolle from a Mycetoma at Tozeur.

It will be obvious that the form of spore formation described above gives rise to a *Thallospore*, which is defined by Vuillemin as follows:—

'The Thallospore is a sporiform element which is really only a portion of the thallus secondarily adapted to the purposes of reproduction.'

The various forms of Thallospores are Blastospores, Arthrospores and Chlamydospores, and it is equally obvious that the spores we are considering are Arthrospores, which are defined as:—

'A thallospore developed by the disarticulation of hyphal elements at first with square ends, which subsequently become rounded off, and have thin walls, which eventually become thickened.'

The classification of the Genus *Madurella* is therefore sufficiently simple. It belong to Fuckel's Fungal Class of the *Fungi Imperfecti*

founded by him in 1869, and to the sub-class *Hyphales* differentiated by Vuillemin in 1910, and to the order *Thallosporales*, also defined by Vuillemin in 1910.

This order is divided into two sub-orders, viz. :—

A. Reproduction by blastospores	Sub-order 1 *Blastosporales* Vuillemin 1911
B. Reproduction by arthrospores	Sub-order 2 *Arthrosporales* Vuillemin 1911

The Arthrosporales, which are known to be parasitic in man, are contained in the genera which may be differentiated as follows :—

A. Yeast-like forms with short hyphae	Genus 1 *Mycoderma* Persoon 1822
B. Without yeast-like forms :—	Genus 2
I. Producing ' Piedra ' on hairs	*Trichosporum* Behrend 1890
II. Producing ' Black Maduromycosis ' ...	Genus 3 *Madurella* Brumpt 1905
III. Producing ' White Maduromycosis ' ...	Genus 4 *Indiella* Brumpt 1906

Genus *Madurella*. A classification of the species of the genus *Madurella* is as follows :—

Genus *Madurella* Brumpt 1905 emendavit Pinoy 1912

Series A. Cultivated and named species :—	
I. Grains (Sclerotia) large, 0·5—1·0 mm. in diameter. In cultures, spores 2-5 microns in diameter ; so-called sclerotes common in depths of media. Animal inoculations : negative. Habitat : Africa	*mycetomi*
II. Grains (Sclerotia) small, the largest rather less than a pin's-head in size. In cultures, spores 2 microns, rarely 5 microns, in diameter ; so-called sclerotes rare and only formed on the surfaces of media. Animal inoculations : positive. Habitat : Africa	*tozeuri*
Series B. Named, but not cultivated, and hence only provisionally placed in the genus :—	
III. Grains (Sclerotia) vary from the size of a millet seed to that of a pea. Habitat : Italy	*bovoi*

As we have already observed above, some investigators consider the *M. bovoi* will prove to be an Aspergillus.

It may be noted that Hatch and Childe describe a case which was a combination of Black and White Mycetomas, but which Musgrave and Clegg consider to be an Actinomycosis.

With regard to Carter's Black Maduromycosis, Semon's researches, in our opinion, show that it is more nearly allied to the fungus which we are about to describe than to Brumpt's genus as defined above, while it is not possible to classify Wright's organism.

But the genera Monosporium (Scedosporium) and Madurella by no means include all the fungi known to cause *Black Maduromycosis*, for Bouffard, in 1905, described a case in Djibouti in which there was no suppuration. The grains were very characteristic, being black in colour and elastic, and breaking when crushed, muriform in appearance, smooth and shiny, and varying in size from a pin's head to a No. 0 shot, and composed of a spirally rolled up mass of hyphae. The pigment is purely superficial, except at a kind of hilum, by which the young mycelial filaments pass out and by their growth increase the size of the grain. The periphery of the sclerote is seen to possess some badly defined granulations, which on careful examination are found to be masses of conidia detached from the conidiophores, while more careful search revealed three heads typical of an Aspergillus, and therefore Brumpt gave the fungus the name of *Aspergillus bouffardi* Brumpt 1905. So far it has not been cultivated, nor have inoculation experiments into monkeys, dogs, cats and gazelle been successful.

Thus the Black Maduromycoses may be arranged provisionally into the following groups :—

A. African Black Maduromycoses :—

I. Brumpt's Black Maduromycosis, found in Somaliland, and caused by *Madurella mycetomi* (Laveran 1902).

II. Nicolle and Pinoy's Black Maduromycosis, caused by *Madurella tozeuri* (Nicolle and Pinoy 1908).

III. Bouffard's Black Maduromycosis, caused by *Aspergillus bouffardi* Brumpt 1905.

B. European Black Maduromycoses :—

IV. Bassini's, Köbner's and Schmincke's Black Maduromycosis, of which the classification of the aetiological fungus is unknown.

V. Bovo's Black Maduromycosis, of which the causal agent has been called *Madurella bovoi* Brumpt 1910, but this must now be accepted with reserve as it has never been cultivated and may not agree with the definition now given for the genus Madurella.

VI. Pepere's Black Maduromycosis, caused by *Monosporium* (*Scedosporium*) *sclerotiale* Pepere 1914.

C. American Black Maduromycoses :—

VII. Wright's Black Maduromycosis, of which the systemic position of the causal fungus is unknown.

VIII. Seheult's Black Maduromycosis, of which the nature of the causal fungus is unknown.

D. Asian Black Maduromycosis :—

IX. Carter's Black Maduromycosis, with a doubt as to the exact nature of the causal fungus.

In addition, Black Maduromycoses have been found in various parts of the world, but the descriptions being clinical, these forms are better recorded in the geographical section of this paper, as there is no indication as to the nature of the causal agent.

Having thus defined the present day condition of knowledge of the Black Maduromycoses as far as the limited literature at our disposal in Khartoum permits us so to do, we will now briefly consider the geographical, zoological and botanical distribution of the disease.

GEOGRAPHICAL DISTRIBUTION

Our variety of Black Maduromycosis occurred in the Anglo-Egyptian Sudan, where the disease was first described by Balfour in 1904, and the northern part of which is hot and arid. He gives the native name for Mycetoma as *Napt Hindi Nabit*, and states that the black variety is most frequently encountered, and that the foot is the part principally affected, while the inguinal glands are often involved. In 1908, Wenyon noted its presence at Bor, which is hot but not arid, while Balfour's researches in 1911 have already been noted in the historical section. According to our enquiries, the word most commonly used by natives in the Sudan is '*En-Nabt*,' which means '*The Growth*.'

In addition to the Anglo-Egyptian Sudan, the following is a list of African places from which cases of Black Maduromycosis have been reported :—Algeria, Tunisia, Somaliland, Madagascar, Transkei (South Africa), Senegal, and the French Sudan.

In Asia the disease is recorded from the Yemen, various parts of India, Ceylon, and possibly from North Borneo.

In America it has been described in the United States by Wright, and in the West Indies by Seheult.

In Europe it has so far only been found in Italy, and Southern Germany.

This distribution, according to political geography, has but little meaning when the object being studied is a fungus, and for further details we turn to plant geography. According to Drude, climatic and local conditions permit the division of the surface of the world into six zones of vegetation, viz. :—The Northern Glacial Zone, the Northern Cold Winter Zone, the Northern Hot Summer Zone, the Tropical Zone, the Southern Hot Summer Zone, and the Southern Cold Zone.

The Black Maduromycoses occur in the Northern Hot Summer Zone, which includes Spain and Italy, North America, the Sahara, Indo-China, Malay Archipelago, the United States (roughly south of Utah), and Mexico. The general characters of this region are :— Very hot summer temperatures with cold nights and no real winter, but with varying rainfall. It contains very dry climates; it also contains wet areas. The Black Maduromycoses are most commonly met with in the dry parts of this area.

The Tropical Zone, which appears to be the real home of these fungi, is generally humid, but contains arid regions bordering upon the preceding. In this zone comes the Anglo-Egyptian Sudan, in the northern or more arid part of which Black Maduromycoses are common, and the same remarks apply to Somaliland, while West Africa is mostly moist.

It also includes the greater part of India, in which the distribution of Mycetoma, according to Boccaro, is interesting.

This observer states that Major Prain divided India into six Floral Regions, viz. :—*India Deserta*, *India Diluvia*, *India Aquosa*, *India Vera*, *India Sub-Aquosa* and *India Littorea*, while Black Maduromycosis is found in only India Deserta and India Vera, and is practically almost absent in other regions.

India Deserta includes the Indus Plain Region, i.e. Sind, Rajputana and the Punjab; while *India Vera* includes the Deccan Region, consisting of the dry but not desert triangle between the

Western and Eastern Ghats, with its apex at Tinnevelly and its base at the borders of the plain of the Ganges.

The white varieties of Mycetoma are also found in this area, but are outnumbered by the Black Maduromycosis, while in *India Deserta* the preponderance of the Black Maduromycoses is even more marked than in *India Vera*.

In Madura and adjoining districts of Tinnevelly, Palmcotta and Coimbatore, situate in *India Vera*, Mycetoma is very common, and the climate is hot and arid.

The Southern Hot Summer Zone includes South Africa, where the disease has been recorded, but where it is apparently rare.

This is as far as the present state of our knowledge permits us to go with regard to geographical distribution, and more research on this part of the subject is required, but from the above it is obvious that heat and aridity are favourable conditions for the fungi which cause Black Maduromycosis.

BOTANICAL AND ZOOLOGICAL DISTRIBUTION

Unfortunately, we are in complete darkness as to the characters which the fungi causing Black Maduromycosis assume when not living in animals or on artificial culture media.

Even with regard to those forms of Black Maduromycosis due to an Aspergillus, we are quite ignorant as to whether this particular fungus lives on soil or on plants.

Having now reviewed the state of knowledge with regard to the distribution of the disease, we will turn to the description of the variety which we have found in the Sudan.

KHARTOUM CASE

We owe the Black Maduromycosis about to be described to the kindness of Dr. Bousfield, Medical Officer of Health of Khartoum and Omdurman, who removed it entire from the sole of the foot of a native boy, where it was found to be lying superficial to the plantar fascia and beneath the skin. No thorn, splinter or foreign body could be discovered in the tissue, although there was a suspicion that it took its origin from such a source.

The boy made a good recovery, and up to the date of writing

(some six months later) there has been no recurrence. There was no ulceration or suppuration, nor were any of the lymphatic glands of the leg enlarged.

MORBID ANATOMY

The pathological anatomy of Black Maduromycosis has been the subject of a fair amount of investigation. Kanthack merely drew attention to the fact that the black masses were always to be found embedded in dense fibrous tissue, while a few pus and granulation cells were to be seen in most cases. In the fibrous wall, yellowish brown or black pigment could be found, while Fuchsin bodies were present in most specimens. Unna's example, obtained from Kanthack, only showed fibrous and some granulation tissue. Boyce and Surveyor drew attention to the presence of small round cells, macrocytes and giant cells surrounding the fungus in cases of Black Maduromycosis. Their microphotographs are, however, mainly devoted to the fungus, while their fig. 22 evidently depicts a very young piece of fungus surrounded by giant cells.

Wright (1898) stated that the nodules consisted of more or less atypical connective tissue, in the cavities of which the granules lay surrounded by polymorphonuclear leucocytes, loose epithelioid cells and cellular detritus. The cavities were lined by either a wall of vascular granulation tissue or by masses of epithelioid and multinucleated giant cells, while these cells closely invested other granules, and outside of this tissue lay lymphoid and plasma cells. He gives four excellent low-power photographs, of which figs. 4, 5, and 6, though older, if examined with a lens, will be seen to agree more or less with Boyce and Surveyor's fig. 22.

Oppenheim's description in 1904 mainly deals with the fungus, but Brumpt's account of the histological changes induced by *Aspergillus bouffardi* covers all the important points, viz.: the polymorphonuclear leucocytes, the lymphocytes, the giant and epithelioid cells, the connective tissue, the cells containing brown pigment and the endarteritis. On Plate XIX, fig. 7, and Plate XX, figs. 1 and 2, he shows appearances resembling those described by Boyce, Surveyor and Wright in a young grain in which the giant cells are situate close to the fungus.

Boccaro, writing in 1909 in general terms for the encapsulated form of both white and black Mycetomas, says:—

> 'The fungal hyphae are surrounded by round cells, held together by a delicate network of fine blood vessels, the cells being located in the meshes of a fibrillar transparent reticulated substance. On the inner side of the group of round cells, between them and the central hyphal mass, is a collection of *finely-granulated débris*, and on the outer side, in most preparations, may be seen large nucleated cells, giant cells, and phagocytes.'

This description, which unfortunately is not illustrated, agrees well with our specimen.

Balfour, in 1911, published photomicrographs of Black Maduromycoses believed to be due to *Madurella mycetomi* and to *Aspergillus bouffardi*, but did not describe them.

Babès (1913) gave a well-illustrated account of Indian Black Maduromycosis, in which he observed far less cells than we have noticed in immediate relationship to the fungus, from which the giant cells were separated by fibrous connective tissue. He drew attention to violet and reddish rounded bodies enclosed in cells.

This, as far as we know, concludes the original observations on the pathological anatomy of Black Maduromycosis of modern date.

In our own case, the tumour described in the clinical section was divided longitudinally into two halves, from one of which the grains were extracted for cultural and other purposes, while the other half was reserved for histological examination.

The tumour measured some 18 millimetres long by 8 millimetres broad by 5 millimetres deep, and was firm to the touch. Fig. 1 shows the general appearance of the growth very slightly magnified. It will be observed to be largely composed of fibrous tissue containing black particles—the grains—and some spaces which are formed by the falling out of some of the black granules during preparation. The spaces demonstrate the character of the lacunae occupied by the grains and their surrounding cells.

Fig. 2 should be examined by means of an ordinary reading lens, when its details can be studied. It will be observed that at the lower part of the photograph lies the fungal mass embedded in cellular tissue. The spaces are artefacts produced in making the section, which otherwise is as natural as possible, i.e. is not bleached or softened in any way. The cracks in the black mass are also artefacts. Around the fungus lies a mass of small cells, and on the

upper and left side of the grain are seen some giant cells, which also occur in other parts but are not in such close relationship to the fungus; then comes some fibrous tissue containing a number of cells, blood vessels and lymph spaces, the last mentioned being situate towards the top of the photograph and being markedly dilated. At the very top of the figure, and only partially shown, comes the dense fibrous connective tissue which is continuous with the dense tissue, depicted in fig. 1, which permeates and surrounds the growth. Therefore the main features of the tumour may be summarised as follows:—

1. Fungus
2. Small cells.
3. Giant cells and large cells.
4. Small cells, connective tissue, blood vessels and lymph spaces.
5. Dense connective tissue.

The description of the fungus will be delayed until the section upon the aetiology, and we will commence our more detailed description with the layer of the small cells.

If the reader examines fig. 7, he will see in the upper part of the photograph a dark area which represents the pigmented fungal mass. Just below this he will observe granular tissue and degenerating cells, while lower down polymorphonuclear leucocytes and some mononuclear cells can be distinguished. The cells are not supported by fibrous tissue, but are separated from one another by granular débris. No blood vessels can be observed.

Fig. 5 depicts in greater detail the degeneration of the cells and the masses of granules which are the product of disintegrated cells.

Fig. 3 shows a number of giant cells, some of which contain fragments of fungal origin. Some large mononuclear cells can also be seen with excentrically placed pale staining nuclei and much granular cytoplasm, which may be vacuolated and may contain the phagocyted remains of the nuclei of the polymorphonuclear cells as portrayed in fig. 8, which shows a normal polymorphonuclear leucocyte and a large phagocyte filled with engulfed nuclei.

The next layer is illustrated by fig. 9, which depicts at the bottom of the photograph small mononuclear cells, many of which possess a fair amount of cytoplasm, which in some instances is

eosinophile. These cells are separated by a variable amount of fine connective tissue, which also supports large lymph spaces and blood vessels. Débris and pigmentary granules can also be seen. A special rare feature of this layer is the presence of mononuclear cells containing one or more eosinophile rounded bodies, which were first observed in this pathological condition by Kanthack, and subsequently by nearly all the other workers on the morbid histology of the Black Maduromycoses, to which they are, however, not confined. Their exact nature is unknown, but they are probably in some way due to the fungus.

On inspecting the upper part of the cellular mass, it will be observed that the white fibrous tissue increases in amount, but is still loose and contains many cells in its meshes, while more externally, and situate at the top of the photograph, is seen the denser and less cellular connective tissue, which is continuous with that separating one fungal mass from another and surrounding the whole tumour. In this connective tissue, cells containing yellowish granules are frequently observed.

There are also many lymph spaces and blood vessels, but the latter at times show signs of endarteritis (fig. 6) or periarteritis (fig. 4), by which means the lumen of the vessel may be considerably diminished or even closed.

It will be observed that the above description agrees in all important points with those given by other authors, though it varies somewhat in details, and we may therefore assume that there is a general similarity as to the histological reaction on the part of the body against the fungi causing the Black Maduromycoses, and, therefore, we will now pass on to consider the nature of the causal fungus which we have obtained from the Khartoum case.

AETIOLOGY

The outstanding feature of the microscopical specimens prepared from this case of Black Maduromycosis is the presence of black granules embedded in the tissues, as depicted in fig. 1. When these black granules are examined by higher powers of the microscope, they are seen to be largely composed of fungal hyphae

embedded in an interhyphal matrix, as is shown in figs. 10, 22, 23 and 24.

The aetiological importance of these black grains and their contained fungus rests upon the fact that they are present in all forms of Black Maduromycosis, and are co-extensive with the disease, while their complete removal, as in the present instance, effects a rapid and complete cure. Animal inoculations with the grains of Black Maduromycosis are, as far as we know, unsuccessful, while the infection of animals by inoculations of cultures has only succeeded with the varieties met with by Pinoy and Pepere.

Therefore the final and convincing proof of the aetiology of the fungus has only been obtained with regard to two varieties, still, for practical purposes, the aetiology of the other known examples also is firmly established.

The various points to be studied with regard to the aetiological factor in the present case may be divided into:—

1. The histology and mycology of the grain.
2. The cultivation of the fungus.
3. The mycology of the cultures.
4. Classification.
5. Animal inoculations.
6. Comparison with the fungi of other Black Maduromycoses.

1. *The Grain.* When a microscopical section, such as that depicted in fig. 22, is examined, it may be noted that it consists of a central or medullary portion, which is light in colour and is surrounded by a thick, dark, radially striated rind or cortex.

On closer examination, the medullary portion is seen (fig. 23) to be composed of segmented, branched, light coloured hyphae, running in various directions, and of roundish, oval, or irregularly shaped thick-walled bodies, which are obviously chlamydospores. Both these structures are embedded in a light sepia coloured homogeneous matrix, which is very hard and cracks when cut with a razor, as is seen in figs. 2 and 22.

The rind or cortex, on examination with the higher powers of a microscope, exhibits fungal hyphae arranged more or less regularly and radially (fig. 10), thus producing the striated appearance shown in fig. 22. Some chlamydospores may also be seen which,

together with the hyphae, are embedded in a hard sepia tinted homogeneous substance.

The pigment in the grain is solely derived from the fungus, as it can be seen in the cultures, and it appears to us probable that the whole matrix is so derived. It can be bleached by Eau de Javelle or by freshly prepared hypobromite of soda, and when so bleached tinges readily with eosine. In the photographs with which this paper is illustrated, we have preferred to show the grain as naturally as possible, and without bleaching.

With regard to the hyphae mentioned above, they may be seen to differ in no material respect from those commonly met with in fungi.

They are septate and branched, while the interseptal segments measure about 2·1 to 2·8 microns in breadth and some 5·6 to 8·0 microns in length. In some instances they appear to be devoid of contents, but, especially in the rind or cortex which is the growing area, they possess eosinophile granular matter. In this position and towards the periphery of the grain, they are often swollen and assume a knob or club-like appearance at the extremities (figs. 20, 22 and 24), which are in close relationship to the leucocytes shown in fig. 22, from which they are sometimes separated by a hyaline eosinophile substance, which may appear as a series of finger-like processes in places where the leucocytes have fallen away from the grain. In the medulla the hyphae may be seen cut transversely, longitudinally or obliquely (fig. 23).

In places these hyphae may be observed to swell considerably, giving rise to larger or smaller thick walled, rounded, oval or irregularly shaped bodies (figs. 12, 13 and 23), which are to be looked upon as intercalary chlamydospores, which apparently become detached from the hyphae when old, and can be seen lying by themselves. The contents of the chlamydospores vary, being sometimes eosine staining granular material, which at other times may be entirely wanting or may have its place taken by a dark sepia tinted substance somewhat resembling the homogeneous matrix, while much more rarely the contents appear in the form of minute clear bodies (fig. 13). The walls of these spores are usually thick, and sometimes very thick (fig. 12), while they may be light in colour or deeply tinted, being then more or less of a sepia colour.

The chlamydospores which we have measured varied between about 7 microns in transverse diameter in the roundish forms to 15·4 by 14·0 microns in the oval varieties.

That these bodies are really chlamydospores may be judged from the photographs, and from the usual definition of such bodies, which is as follows:—

'An intermediate or terminal spore larger than an ordinary hypha which, without becoming isolated, undergoes a kind of encystment with the formation of a thick, and sometimes coloured, wall, containing cytoplasm loaded with food material.'

The next point to be considered is the mycological position of the grain, and it appears to us the only fungal structure which at all resembles it is a 'sclerotium,' which is defined by De Bary as follows:—

'The name *sclerotium* has been given to certain thick tuber-like bodies, formed on the primary or filamentous mycelium, which proceeds from the germinatory spore; these, which are storehouses of reserve material, become detached from the mycelium when their development is complete and usually remain dormant for a considerable time, and ultimately expend their reserve material in the production of shoots, which develop into sporophores.'

The grains we are considering do not agree well with this definition. They may be storehouses of reserve material, as the matrix may be of this nature, but it appears to us more probable that it is protective in character, because a grain, so far as our experience goes, will not grow unless placed under extremely advantageous food conditions, e.g. being placed in glucose peptone, thus giving no indication that it contains food materials in itself, as is the case with the sclerotium of the ergot of rye.

Again, it is not a part of the mycelium, but is the mycelium itself, and can grow, which a sclerotium is not supposed to do, while its rind is the actively growing part, though in a sclerotium it should be composed of cells with sclerosed cell walls which are dark in colour and have little solid cellular contents.

It appears to us to be probable that it is from the clubs of the rind or cortex that the hyphae of the puff-ball which forms in glucose peptone may develop, though this is a pure supposition on our part, as we were not in possession of sufficient black grains to test this point, nor, as far as we know, has it ever been investigated by any one up to date.

Moreover, we are not acquainted with any literature describing 'chlamydospores' inside a 'sclerotium.'

We therefore conclude that, although the grain may superficially resemble a sclerotium, it differs therefrom in that it is *the mycelium*, and not a detached portion formed in a mycelium; it can grow, its cortex is not composed of protecting cells, and it is doubtful whether it contains much reserve food material, while finally it does not produce sporophores but a mycelium, which only after a time and after much growth forms chlamydospores and other spores.

We therefore believe that the sclerotium is not homologous with the grain, and we consider that this term should be used in a mycological sense, being called '*granum*,' which may be defined as follows:—

> 'The name "*granum*" has been given to certain differently coloured bodies of varying consistence, size and shape which are composed of hyphae, and sometimes chlamydospores, embedded in a matrix, and which, on germination, give rise to mycelial filaments, and are found in mycetomas.'

Although there is a marked morphological and developmental difference between a 'sclerotium' and a 'granum,' it must be admitted that they both serve the same physiological function in that they are protective and keep alive the growing elements of the mycelium under adverse circumstances.

2. *Cultures.* When a grain is incubated in glucose peptone medium at 30° C. under aërobic conditions in an incubator it sprouts, producing a large number of white hyphae, which form a structure resembling a puff-ball having the remains of the black grain in the centre. These white hyphae grow until they reach the walls of the glass tube and the surface of the medium upon which they form a skin, whilst the fluid itself acquires a dark reddish brown colour, due to the pigment derived from the fungus.

When placed upon maltose agar, under similar conditions, it produced hyphae, which formed a growth which finally obscured all trace of the original grain. This growth, which is depicted in fig. 14, is black in colour, and has a central elevation surrounded by a depression which separates it from a grooved, raised plateau, which has a slight fringe. At first the growth was greyish white, but gradually it became darker and darker, while the medium also increased its own naturally dark tint. To be exact, the colour of the

growth agreed with Ridgway's Standard Colour 'Dusky Drab,' as depicted in his Plate XLV, 9, ORO, K.

From this culture on maltose agar all the sub-cultures were made.

With regard to its biological characters, the fungus grew under aërobic conditions at 22° C., 37° C., and 40° C., as well as at 30° C., but it did not grow at 60° C. nor anaërobically.

The varying characters of its growths at different temperatures, but on the same medium (Sabouraud's maltose agar) and for the same number of days (eleven), are depicted in figs. 15 at 22° C., 17 at 30° C., 18 at 37° C., and 21 at 40° C.

It will be observed that 30° C. is the optimum temperature, and also that the originally black growth has become covered with a greyish 'duvet.'

When grown in a watch glass, by the method described by Chalmers and Marshall, it produced a growth of which fig. 11 is a photograph, taken on the ninth day. The grooves on the plateau are not so marked as when grown in a test tube. A peculiarity of these cultures is a grey fluffy appearance, which forms on the surface of the black growth when exposed freely to the air in watch glasses, Kitasato flasks and uncapped test tubes. In order to demonstrate the fluffy appearance, fig. 16 is reproduced, which is a photograph of the same culture as that represented in fig. 11, but magnified four times.

Fig. 19 shows a similar growth on maltose agar, but produced in the protection of a Kitasato flask. In all these cultures the black pigment was very marked, as could be seen when the deep surface of the growth was examined.

Fig. 34 shows another growth on maltose agar after fifteen days in a capped tube at 30° C. It will be observed that the greyish fluff is nearly absent, although it is present to some extent in the white marking on the upper part of the plateau and in the upper part of the fringe. The central knob is represented by an elevated, bare, crumpled transverse ridge, while the plateau is nearly black, though it shows groovings, while the fringe is reduced to a mere margin in the lower part, though more marked in the upper portion of the photograph, where it looks rather elevated, which was due to the fluffy hyphae.

On glycerine agar, glucose agar, and agar agar the growths

resemble that produced on maltose agar. On ordinary agar agar, in eighteen days, it formed a growth with a dark centre and a white periphery. Glucose agar growth in eighteen days resembles that of the maltose agar, but there are at first more white hyphae.

On blood serum it produces the appearance depicted in fig. 38; there being no liquefaction of the medium into which the black growth sunk somewhat and subsequently developed a slight duvet.

The development in a gelatine stab at 22° C. was peculiar, in that a growth formed in the stab just below the surface, and spreading upwards and outwards reached the surface, where it produced a white growth placed superficially to a dark feltwork below which the medium became tinged with a reddish brown colour. On this white surface there appeared a number of small hard black bodies (fig. 29), which upon microscopical examination were found to be composed of dark walled Chlamydospores lying crowded together in masses and having a quantity of free pigment between them (fig. 41). Much as these resemble grains in appearance and also in consistence, they are morphologically very different, being merely collections of chlamydospores. There was no growth in the depth of the stab, nor was there any liquefaction of the gelatine.

The growth upon potato at 30° C. is depicted in fig. 28, when it is observed not to be characteristic, but is seen to be puckered, black and fluffy. Later, after eighteen days (fig. 43) it formed a wrinkled black growth with a black central knob. The potato was stained black around the growth, but was not eroded. In infusions of potato it grew well, forming puff-balls, and eventually a skin on the surface in which black masses appeared which were of the same nature as those seen in gelatine. There was no reduction of Fehling's solution by this growth, indicating that no sugar was formed by the fungus, while tincture of iodine produced the usual starch reaction.

On carrot it grew out into a dark mass covered by a greyish fluff (fig. 25). Cheese and lard were tried, and found to be unsuitable for cultivation purposes, as the fungus failed to grow, although it apparently did not die.

In litmus milk it formed a superficial white skin which was pigmented on its deeper parts, but it did not give rise to acid nor

did it form a coagulum, though it cleared the solution, which took on a port wine tint, while it produced a flaky precipitate which fell to the bottom of the tube.

3. *Mycology.* All growths show septate branched hyphae of varying diameter, from 2·8 to 1·4 microns, but in very old cultures (when all the hyphae are dark) they may measure 4·2 microns (figs. 26, 27, 30 and 31). These hyphae are pale when young (figs. 27 and 31), but are dark coloured when older (fig. 30), taking on a greenish black tinge. The length of an interseptal segment varies very considerably, and perhaps 12·6 microns may be considered as an average, but much less than this have been observed, while segments reaching 28 microns in length have been noted in the dark coloured hyphae. In most of the older cultures, clear or dark coloured thick walled chlamydospores (14 × 11·3 microns) may be observed, but they are especially marked in the black masses present on the surface growths on potato infusion and nutrient gelatine (figs. 29 and 41).

The surface growth on old potato infusions, however, produced another feature, viz., that depicted in figs. 26, 27, 30 and 31, which, if examined, are seen to show a spore formation of a different nature to that of a chlamydospore. These spores are seen to be produced at the ends of hyphae or on stalks which look like sporophores, or to be situate pleurogenously, with or without short stalks, on the main hyphae, vide figs. 27 and 31. The same features, but not so marked, are to be seen in fig. 42, which is taken from an old maltose agar growth.

The spores are noted not to be rounded, as is the case with conidia, but to be truncated by the septa which cut them off from the hyphae, and thus they are not round but oval.

The spore-bearing hyphae are generally branched, vide fig. 31, and often the segment below the spore produces one (fig. 26) or, more rarely, two lateral projections, which make an appearance as though there were three spores, i.e. one central and two lateral.

Another remarkable feature of these spores is their persistence in adhering to the hyphae. Whereas in teased preparations from fungal growths with spores it is customary to find many detached spores lying in the field of vision, it is rare in the present case to

see a detached spore, even when the preparation has been roughly handled on purpose. The spores are unicellular and pale when young.

Black pigment particles may be observed lying between the hyphae.

No sign of any sexual reproduction was observed in our preparations.

4. *Classification.* It is obvious that as the fungus in question is a parasite with filaments clearly septate, reproducing by spores and obvious sexual cells, it belongs to the *Eumycetes* of Schröter, and not to the *Phycomycetes* of De Bary. As the spores are not contained in asci or basidia, it is equally manifest that it must be placed in Fuckel's class called *Fungi Imperfecti.*

As no accessory fructifications can be seen, and as reproduction appears to be by means of spores situate on hyphae, it must be placed in Vuillemin's sub-class *Hyphales.* The further classification depends on the view taken of the spores depicted in figs. 26, 27, 30 and 31.

As they do not appear to be capable of forming new spores or hyphae while still attached to the parent mycelium, they would be classed as conidia, of which there are two main types, viz., the Aleuriospore and the Conidium Verum.

> The *Aleuriospore* is a conidium which is characterised by being truncated by the septum which separates it from its parent hypha, to which it is so closely united that it is only set free by destruction of that structure.
>
> The *Conidium Verum* is not truncated, but, on the contrary, is constricted, thus producing a rounded appearance, and is attached to its parent hypha by only a restricted area of its rounded periphery, from which, as a rule, it is easily detached.

The two main features of the Aleuriospore, viz., its broad attachment to the parent hypha and its persistent adherence thereto, are well exhibited in figs. 26, 27, 30 and 31, which are teased preparations.

We, therefore, conclude that these spores are *Aleuriospores*, and with this deduction we are now in a position to proceed with an analysis of Vuillemin's classification of the *Hyphales.*

As the fungus under consideration does not possess a mycelium

composed of bacilliform, but, on the contrary, of broad septate hyphae, it does not belong to the order *Microsiphonales*, and as its method of reproduction is by means of *Conidia*, it belongs to Vuillemin's order of the *Conidiosporales*, which is divided into five sub-orders as follows :—

A. Conidium imperfect being in the form of an Aleuriospore	Sub-order 1 *Aleuriosporineae* Vuillemin 1911
B. Conidium perfect :—	Sub-order 2
I. True conidiophores absent	*Sporotrichineae* Vuillemin 1910
II. True conidiophores present :—	Sub-order 3
a. Conidia borne on sporophores	*Sporophorineae* Vuillemin 1910
b. Conidia borne on phialides :—	Sub-order 4
1. Prophialides absent	*Phialidineae* Vuillemin 1910
2. Prophialides present	Sub-order 5 *Prophialidineae* Vuillemin 1910

From this table it is apparent that the fungus described above belongs to sub-order 1, *Aleuriosporineae*, which is classified by Vuillemin as follows :—

A. Conidiophores absent	Family 1 *Aleurismaceae* Vuillemin 1911
B. Conidiophores present	Family 2 *Monotosporaceae* Vuillemin 1911

Figs. 26, 27, 30 and 31 show that true Conidiophores are absent, and that the Aleuriospores are borne either acrogenously or pleurogenously on hyphae, from which they are separated by means of a septum which truncates the spore, and therefore does not produce the constriction associated with the separation of a Conidium from a Conidiophore.

The genera of the family *Aleurismaceae* may be recognised by the following table :—

A. Hyphae pale :—	Genus 1
I. Hyphae very short, with sporogenous apparatus but little distinct from the mycelium ...	*Myceliophthora* Costantin 1894

II. Hyphae elongate, with sporogenous apparatus distinct from the mycelium. Fertile hyphae branched :—	
a. Aleuriospores smooth, small and acro-pleurogenous :—	Genus 2 *Aleurisma*
1. Aleuriospores coloured	Link 1809
2. Aleuriospores pale	Genus 3 *Corethropsis* Corda 1839
b. Aleuriospores spiny, large and acro-genous :—	Genus 4 *Mycogone*
1. Aleuriospores appendiculate	Link 1809
2. Aleuriospores not appendiculate ...	Genus 5 *Sepedonium* Link 1809
B. Hyphae dark :—	
a. Aleuriospores small (generally 6 × 4, rarely 11 × 5, microns), become dark, situate acro-pleurogenously on dark or light coloured hyphae	Genus 6 *Glenospora* Berkeley and Curtis 1876
b. Aleuriospores large (11-14 microns), remain hyaline, situate acrogenously on hyaline hyphae at the base of sterile dark hyphae .	Genus 7 *Botryotrichum* Saccardo and Marchal 1885

It will be observed that the fungus under consideration belongs to the section with dark hyphae, and is closely related to the genus *Glenospora* Berkeley 1876, while it is easily differentiated from *Botryotrichum* Saccardo and Marchal 1885 by the size and arrangement of its Aleuriospores.

We therefore consider that in all probability the fungus which we are considering should, at all events, *provisionally*, be placed in the genus *Glenospora*, because its Aleuriospores are small and are situate acro-pleurogenously on dark or light coloured hyphae.

If the older method of classification is adopted it still belongs to the *Fungi Imperfecti* ; Order Moniliales (*Hyphomycetaceae* Martius 1817), because its hyphae are more or less developed and cobwebby, or more or less compact, and never enclosed in a pycnidium, and typically superficial ; Family Dematiaceae Fries 1832, because its hyphae are dark or black, cobwebby, loose, usually rigid, conidia typically dark and concolorous, but sometimes the hyphae are dark and conidia clear or vice versâ. As the hyphae are manifest and distinct from the conidia it comes under the *Macronemeae*, and as its conidia are subhyaline or dark, not in chains but inserted irregularly, while in its growths it tends to form a crust, it again comes down to the genus *Glenospora*.

It also agrees well with Lindau's definition of the genus as given in Engler and Prantl's Pflanzenfamilien, which reads as follows:—

'Hyphen und Conidienträger eine schwarze kruste bildend. Conidienträger septiert verzweigt. Conidien endständig und seitenständig meist einzeln, lange anhängend, kugelig, ziemlich gross, grünschwarz.'

It does not quite agree so well with Saccardo's definition, which is:—

'Hyphae biogenae in crustam atram intextae, variae ramosae septatae. Conidia ramulus diu haerentis, globosa majuscula, levia.'

but this definition is only written to include *G. curtisii* and *G. ramorum*. As we have been unable to obtain any specimens of other species of Glenospora with which to compare the fungus which we are considering, we have been compelled to trust to descriptions and illustrations, which are often misleading, and therefore we only *provisionally* classify it as a *Glenospora*.

We now come to the consideration of the species, and we find that Saccardo, in 1886, in Volume IV of his 'Sylloge Fungorum,' only lists two species, viz.: *G. curtisii* Berkeley and Desmond, which is apparently the type, and *G. ramorum* (Schweinitz 1822), while Lindau, writing in 1900, stated that there were four known species, but the literature available in Khartoum does not indicate what these other two species may be, on the other hand, we are well acquainted with the literature pertaining to the human parasite *Glenospora graphii* (Siebenmann 1889), found in cases of Otomycosis and Keratomycosis.

There is one difference which has impressed us considerably, and this is the variation in the dimensions of the spores, thus Saccardo gives the measurements of the Aleuriospores of *G. curtisii* as being 10 to 12 microns in diameter, while Landrieu gives those of *G. graphii* as 6 to 6·7 × 4 to 5 microns, and only exceptionally 11 × 5 microns, so that the average transverse diameter would be about 4 to 5 microns.

The measurements of the spores in our specimens are about 4 to 5 × 3 to 4 microns, which gives a transverse diameter of about 3 to 4 microns.

We also observed that Lindau uses the term 'ziemlich gross' for the spores of the genus.

The Aleuriospores of our specimens are smaller than those of

G. graphii, but, apart from this, there appears to be very little morphological difference between the two fungi. We have inoculated our fungus into the anterior chamber of the eye in rabbits with negative results, as has Landrieu with *G. graphii*. We have never seen any illustrations of cultures of *G. graphii*, but the only differences which we can find are that *G. graphii* only slightly modifies milk (in what way the milk is changed is not stated), and the colonies remain white or grey much longer on serum than on other media, while with our fungus milk is profoundly altered, as described above, and a black growth is quickly formed on serum.

The only important difference is that in man *G. graphii* produces only Otomycosis and Keratomycosis, while our fungus produces a Mycetoma; we therefore conclude that they are different species, and therefore name our fungus *Glenospora khartoumensis* Chalmers and Archibald 1916.

It appears to us therefore to be possible to divide the species of Glenospora into two groups, as follows:—

A. Aleuriospores large, usually measuring ten or more microns in diameter:—
Parasitic on Plants ... ... { 1. *curtisii* / 2. *ramorum*

B. Aleuriospores small, usually measuring five or less microns in diameter:—
I. Parasitic in Man, causing Otomycosis and Keratomycosis ... ... 3. *graphii*
II. Parasitic in Man, causing Black Maduromycosis ... ... 4. *khartoumensis*

5. *Animal Inoculations.* It may briefly be stated that experiments with the grains or with cultures in monkeys, rabbits and pigeons intraperitoneally, subcutaneously and deep into the tissues in various parts of the body, associated with or without the introduction of a thorn, and into the anterior chamber of the eye, have one and all proved to be complete failures, the fungus in all cases disappearing without producing any effect. The experiments with cultures were repeated several times, always with the same result.

We suspect that even man must be fairly resistant to the infection, because many native people must be infected through cuts and scratches, after which the fungus must be completely destroyed, while it must be relatively rare for a Mycetoma to develop,

otherwise there would be far more cases of the disease that there are, especially in a country like this, where thorns grow on many plants and where some native tribes go about naked.

Comparisons. It will be sufficiently obvious that *G. khartoumensis* is very separable from the various species belonging to the genus *Madurella* Brumpt 1905, emendavit Pinoy 1912, because the latter belongs to the Hyphal order *Thallosporales* Vuillemin 1910, while the former comes under the Hyphal order *Conidiosporales* Vuillemin 1910.

Therefore the Khartoum Black Maduromycosis can be readily differentiated from :—

1. Brumpt's Black Maduromycosis;
2. Nicolle and Pinoy's Black Maduromycosis;

but it will also be obvious that if the three fungi had not been cultivated then differentiation would have been difficult.

With regard to Pepere's Maduromycosis, we note that *G. khartoumensis* belongs to the sub-order *Aleuriosporineae*, while Pepere's fungus *Monosporium sclerotiale* or *Scedosporium sclerotiale* belongs to sub-order *Sporophorineae*, as set forth above, because its spores are true conidia borne on true conidiophores which are of the type of a sporophore (see Chalmers and Archibald, 'The Fungi Imperfecti in Tropical Medicine'), and therefore the distinction between the two types can be made, but with more difficulty than in the preceding cases.

From Bouffard's Black Maduromycosis it can be separated by the absence of aspergillar heads in the microscopical sections.

It cannot be distinguished at present from Bassini's Maduromycosis, as there is insufficient knowledge with regard to this form.

With regard to Wright's Black Maduromycosis, in fig. 12 of Plate XL, he illustrates by a photograph a culture on potato obtained from the black grains, which is altogether different from the photograph of *G. khartoumensis* as shown in fig. 28, Plate III, of the present paper, so that it seems probable that his Maduromycosis is different from the Sudan variety.

We now come to a much more difficult differentiation, viz., that from Carter's Black Maudromycosis, which would have been impossible without Semon's most valuable and brilliant researches of last year.

It is not our intention to enter into a detailed or mycological description of the cultures which, by the kindness of Semon, we have been able to make from his original growth, as he would have done so himself if not employed on active military service for his country. We merely desire to invite the reader's attention to the cultural differences depicted in figs. 33 and 34, figs. 35 and 36, figs. 39 and 40, and figs. 37 and 38 which represent Semon's fungus and *Glenospora khartoumensis* grown under similar conditions and on the same media.

We think that these illustrations are sufficient, without further trespassing upon Semon's kindness, to show that there is a difference between the two fungi, and this is the only point which concerns us.

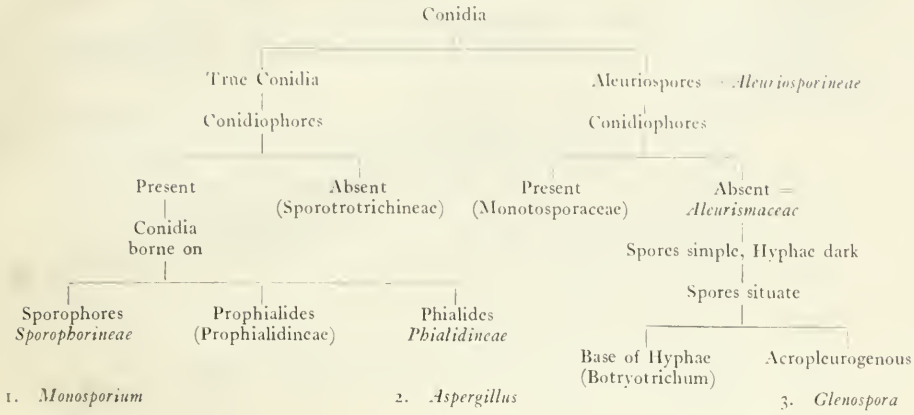
We therefore submit that *G. khartoumensis*, and hence the variety of Sudanese Black Maduromycosis which we have just described, can be differentiated from Semon's Asian Black Maduromycosis (which we believe will be found to be one of Carter's Asian Black Maduromycoses), from Brumpt's African Black Maduromycosis, from Nicolle and Pinoy's African Black Maduromycosis, from Bouffard's African Black Maduromycosis, and from Pepere's Black European Maduromycosis, while the data given for Bassini's, Bovo's, Schmincke's European and Wright's American Black Maduromycoses are insufficient for purposes of definition.

We therefore believe that the Maduromycosis which we have just described is *a new form of African Black Maduromycosis*.

The following diagnostic table of the classifiable fungi found in Black Maduromycosis up to date may perhaps be useful:—

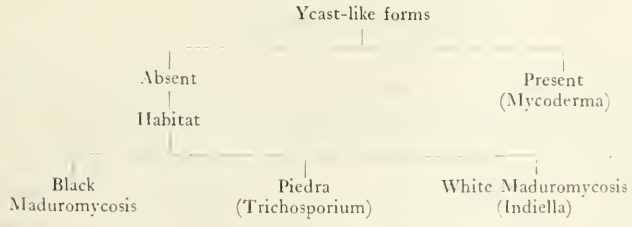
Diagnostic Table I of the Cultivated Fungi in Black Maduromycosis

Hyphae septate, reproduction asexual by spores, sexual usually absent = *Eumycetes*
Spores not in asci or basidia = *Fungi Imperfecti*
Accessory fructifications absent = *Hyphales*
Mycelium not bacilliform (i.e. not belonging to Actinomycosis)
Spores = Conidia = *Conidiosporales*



Diagnostic Table II of the Cultivated Fungi in Black Maduromycosis

Hyphae septate, reproduction asexual by spores, sexual usually absent = *Eumycetes*
Spores not in asci or basidia = *Fungi Imperfecti*
Accessory fructifications absent = *Hyphales*
Mycelium not bacilliform (i.e. not belonging to Actinomycosis)
Spores—Thallospores = *Thallosporales*
Thallospores—Arthrospores = *Arthrosporales*



SUMMARY

We believe that we have cultivated a fungus, which we name *Glenospora khartoumensis* Chalmers and Archibald 1916 from a case of Black Maduromycosis occurring in Khartoum in the Anglo-Egyptian Sudan, and we further consider that this is nearly allied to, but not identical with, the fungus grown by Semon from a case of Black Maduromycosis coming from India.

The known causal organisms of African Black Maduromycosis are therefore:—

I. Brumpt's Black Maduromycosis, found in Somaliland and caused by *Madurella mycetomi* (Laveran 1902).

II. Nicolle and Pinoy's Black Maduromycosis, caused by *Madurella tozeuri* (Nicolle and Pinoy 1908)

III. Bouffard's Black Maduromycosis, caused by *Aspergillus bouffardi* Brumpt 1905.

IV. A Sudan Black Maduromycosis, caused by *Glenospora khartoumensis* Chalmers and Archibald 1916.

The European, American, and Asian Black Maduromycoses have already been summarized.

Diagnosis can only be effected by finding the black grains in the tumour or discharge therefrom, and then cultivating and studying the mycology of the fungus obtained from these grains. So far, the only curative treatment is excision of the growth, which when small can be easily effected, but when large may require an extensive operation or amputation of the affected member. It is important to remember that in some Black Maduromycosis the glands may become affected, and it may be necessary to remove these if enlarged.

ACKNOWLEDGMENTS

Our grateful thanks are due to Dr. Bousfield, Medical Officer of Health of Khartoum, for providing us with the specimens which we have studied; to Dr. Semon, for so generously sending us a growth from his Indian Black Maduromycosis; to Professor Saccardo, for kindly replying to our enquiries; to Professor Vuillemin, for sending us copies of his valuable publications.

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EXPLANATION OF PLATES

Most of these photographs may be examined advantageously by means of a lens, as they are all made from unbleached specimens.

Plate IV

Fig. 1. *Black Maduromycosis.* Longitudinal section through the length of the growth. The black grains can be seen, and also the spaces from which some, with their surrounding cells, have fallen out. × 1·5 diameters. Photograph.

Fig. 2. Section through a grain, as depicted in fig. 1, showing the relationship of the fungus to the surrounding tissues. × 95 diameters. Photomicrograph.

Fig. 3. Giant cells containing fungal tissue. × 140 diameters. Photomicrograph.

Fig. 4. Endarteritis. × 285 diameters. Photomicrograph.

Fig. 5. Degenerating cells and granules in the layer just external to the fungus. × 930 diameters. Photomicrograph.

Fig. 6. Periarteritis. × 650 diameters. Photomicrograph.

Fig. 7. Healthy cells lying just external to the fungus, which only shows as the dark mass in the upper part of the section. × 290 diameters. Photomicrograph.

Fig. 8. A polymorphonuclear leucocyte and a phagocyte, enclosing many nuclei from disintegrated polymorphonuclear cells. × 1,040 diameters. Photomicrograph.

Fig. 9. External cellular layer with supporting fibrous tissue and the innermost portion of the fibrous coat. × 155 diameters. Photomicrograph.

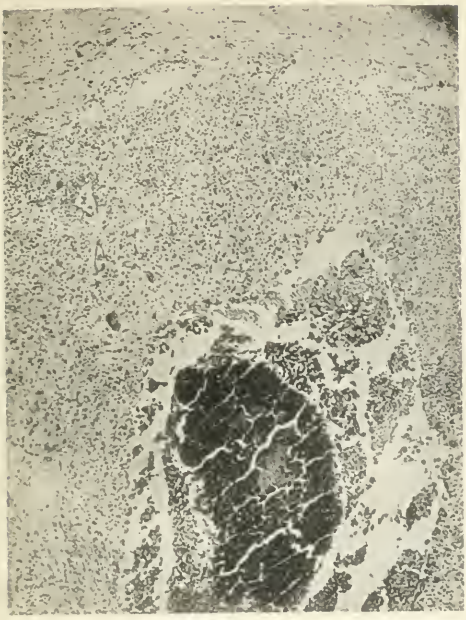


Fig. 2

Fig. 1

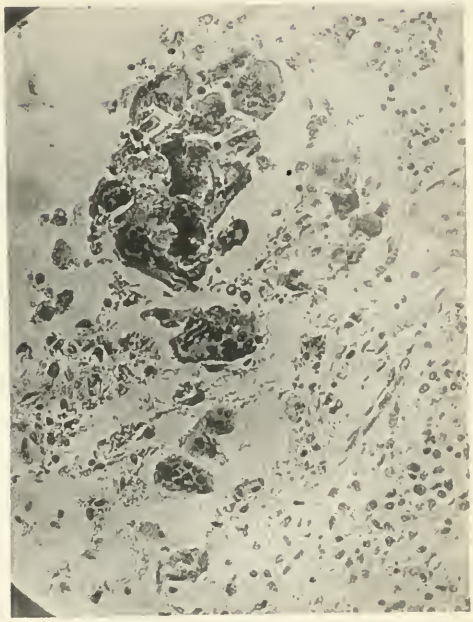
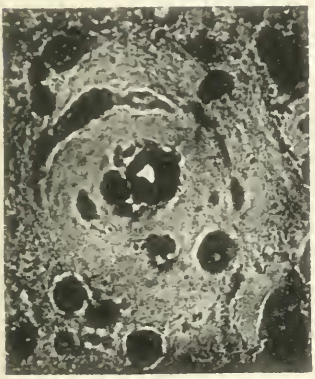


Fig. 3



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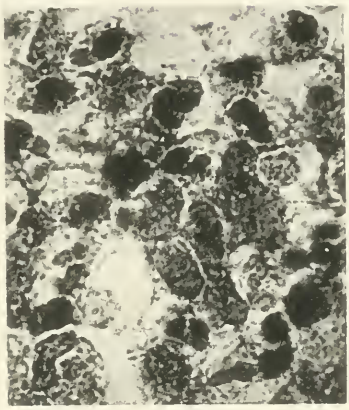
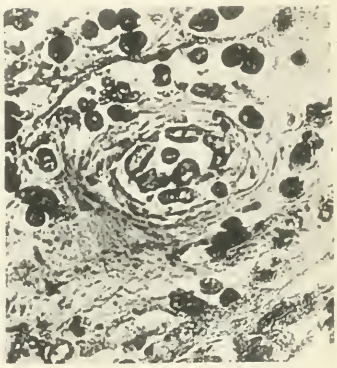


Fig. 5



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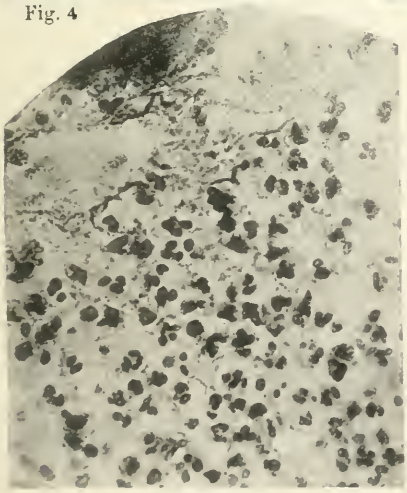

Fig. 7

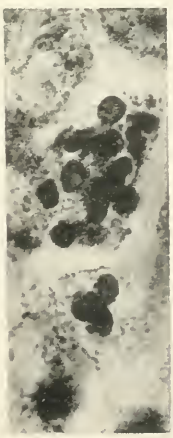

Fig. 8

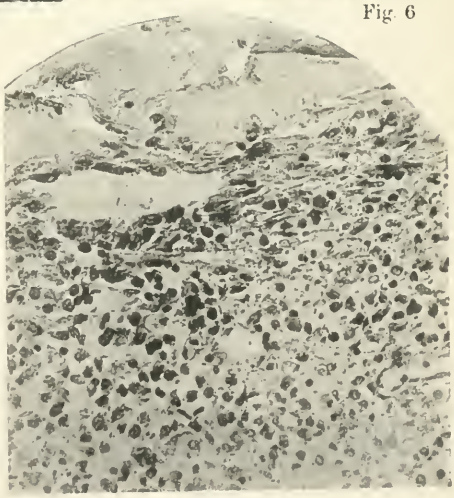

Fig. 9

A SUDANESE MADUROMYCOSIS

Plate V

Fig. 10. Grain showing fungal hyphae in cortical portion. × 250 diameters. Photomicrograph.

Fig. 11. Culture on Sabouraud's maltose agar (watch-glass method) for eight days at 30° C. Natural size. Photograph.

Fig. 12. Grain showing chlamydospores. × 730 diameters. Photomicrograph.

Fig. 13. Chlamydospore containing hyaline bodies. × 985 diameters. Photomicrograph.

Fig. 14. Primary growth from a grain on Sabouraud's maltose agar for twenty-three days at 30° C. Natural size. Photograph.

Fig. 15. Growth on Sabouraud's maltose agar at 22° C. for eleven days. Natural size. Photograph.

Fig. 16. The same growth as that depicted in fig. 11, but magnified 4 diameters. Photograph.

Fig. 17. Growth on Sabouraud's maltose agar at 30° C. for eleven days. Natural size. Photograph.

Fig. 18. Growth on Sabouraud's maltose agar at 37° C. for eleven days. Natural size. Photograph.

Fig. 19. Culture on Sabouraud's maltose agar in a Kitasato flask for eight days at 30° C. Natural size. Photograph.

Fig. 20. Club-like hyphae as seen in fig. 10, but magnified 780 diameters. Photomicrograph.

Fig. 21. Growth on Sabouraud's maltose agar at 40° C. for eleven days. Natural size. Photograph.

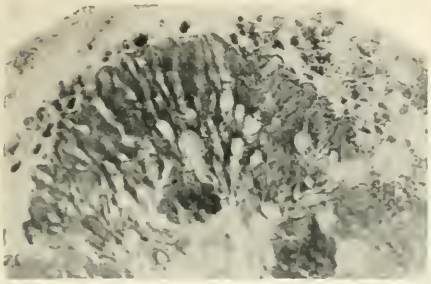

Fig. 10

Fig. 11

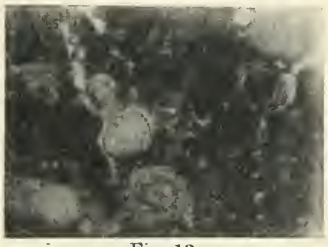

Fig. 12

Fig. 13

Fig. 14

Fig. 15

Fig. 16

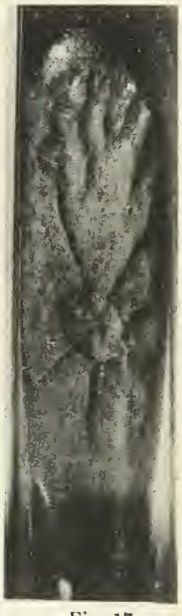

Fig. 17

Fig. 18

Fig. 19

Fig. 20

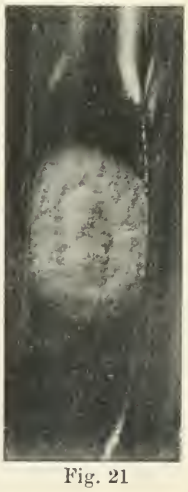

Fig. 21

A SUDANESE MADUROMYCOSIS

Plate VI

Fig. 22. Transverse section of the grain, of which a portion is depicted in fig. 10. × 140 diameters. Photomicrograph.

Fig. 23. Central portion of an unbleached grain to show hyphae and chlamydospores. × 470 diameters. Photomicrograph.

Fig. 24. Part of the cortex depicted in fig. 10 to show a cortical hypha with bulbous peripheral end. × 580 diameters. Photomicrograph.

Fig. 25. Growth on carrot for four days at 30° C. Natural size. Photograph.

Fig. 26. Aleuriospores, sessile on parent hyphae, and on branches, from growth in potato infusion. Living specimen. × 1,370 diameters. Photomicrograph.

Fig. 27. Aleuriospores, taken as naturally as possible to show their grouping. Note that they are sessile on the sides and at the ends of hyphae. Note that no spores are seen detached from the hyphae, even though the specimen was teased. Living specimen. × 400 diameters. Photomicrograph.

Fig. 28. Growth on potato for six days at 30° C. Natural size. Photograph.

Fig. 29. Surface view of gelatine growth showing black masses of chlamydospores. Natural size. Photograph.

Fig. 30. Aleuriospores at the end of hyphae, and a dark tinted segmented hypha showing the dark coloured and light coloured hyphae lying side by side. Living specimen. × 800 diameters. Photomicrograph.

Fig. 31. The branching of a fertile hypha bearing Aleuriospores. All the branches cannot be shown exactly in the same focal plane, but all details can be made out by careful examination, and the photograph is thought by us to be better than a drawing. Living specimen. × 750 diameters. Photomicrograph.

Fig. 32. Young unpigmented chlamydospores. Living specimen. × 730 diameters. Photomicrograph.

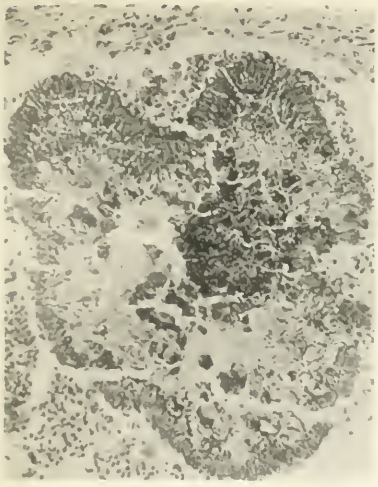

Fig. 22

Fig. 23

Fig. 24

Fig. 25

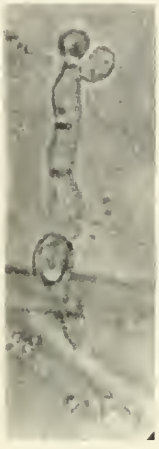

Fig. 26

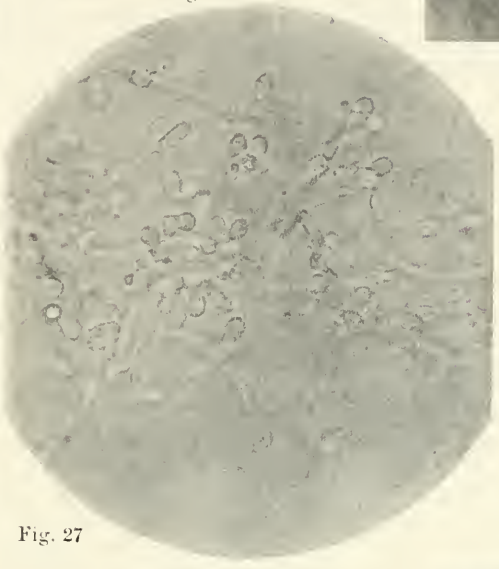

Fig. 27

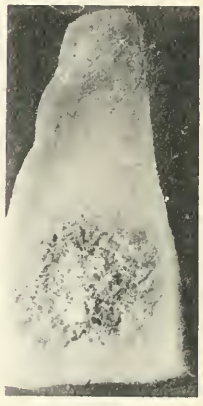

Fig. 28

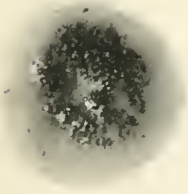

Fig. 29

Fig. 30

Fig. 31

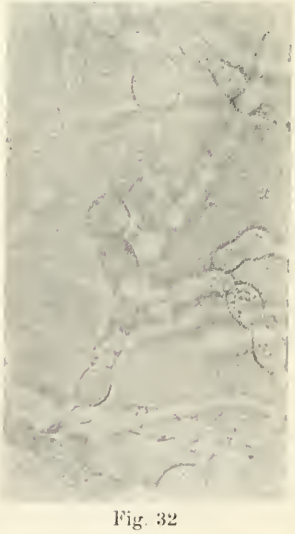

Fig. 32

A SUDANESE MADUROMYCOSIS

Plate VII

Fig. 33. *Semon's Indian Black Maduromycosis.* Photograph of original culture sent to Khartoum in a capped tube, and apparently on glucose agar. Natural size. Photograph.

Fig. 34. *Khartoum Black Maduromycosis.* Grown in a capped tube for 16 days at 30° C. on maltose agar. Natural size. Photograph.

Fig. 35. *Semon's Indian Black Maduromycosis.* Sub-culture made on clear maltose agar in Khartoum from the growth depicted in fig. 33, after twelve days' growth in an uncapped tube at 30° C. Natural size. Photograph.

Fig. 36. *Khartoum Black Maduromycosis.* Grown under exactly similar conditions as the culture depicted in fig. 35 after twelve days' growth. Natural size. Photograph.

Fig. 37. *Semon's Indian Black Maduromycosis.* Sub-culture on inspissated ox-blood serum after twelve days' growth in an uncapped tube at 30° C. Natural size. Photograph.

Fig. 38. *Khartoum Black Maduromycosis.* Grown under similar conditions to fig. 37, and on the same medium. Natural size. Photograph.

Fig. 39. *Same growth as that depicted in fig.* 35, but grown for fourteen days. Photograph.

Fig. 40. *Same growth as that depicted in fig.* 36, but grown for fourteen days. Photograph.

Fig. 41. *Khartoum Black Maduromycosis.* Chlamydospores from growth on gelatine. × 520 diameters. Photomicrograph.

Fig. 42. *Khartoum Black Maduromycosis.* Growth on maltose agar, showing Aleuriospores. × 310 diameters. Photomicrograph.

Fig. 43. *Khartoum Black Maduromycosis.* Growth on potato for sixteen days at 30° C. Natural size. Photograph.

Fig. 33

Fig. 34

Fig. 35

Fig. 36

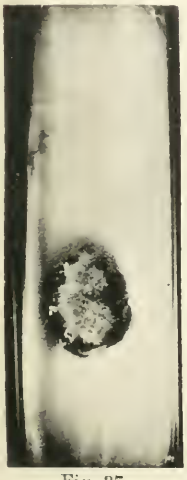

Fig. 37

Fig. 38

Fig. 39

Fig. 40

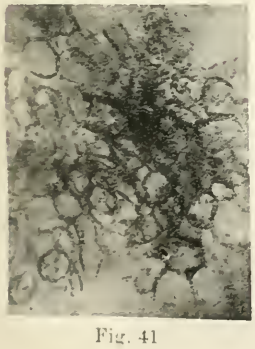

Fig. 41

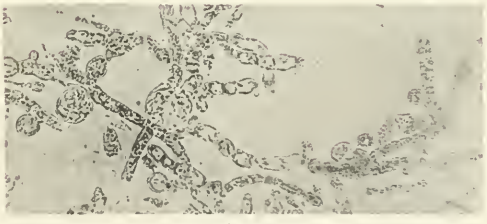

Fig. 42

Fig. 43